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POTENTIAL-DEPENDENT MEMBRANE CURRENT DURING THE ACTIVE TRANSPORT OF IONS IN SNAIL NEURONES

BY P. G. KOSTYUK, O. A. KRISHTAL AND V. I. PIDOPLICHKO

From the Department of General Physiology of the Nervous System, Bogomoletz Institute of Physiology, Academy of Sciences, Kiev, U.S.S.R.

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SUMMARY

1. The membrane current caused by the iontophoretic injection of sodium into giant neurones of the snail *Helix pomatia* was investigated under a long lasting voltage clamp. The inhibition of this current by ouabain (10^{-4} M) and by cooling to $+7°$ C confirmed its link with the active transport of ions. Therefore this current is called the pump current.

2. Over the range of membrane potential -40 to -100 mV the changes in the steady current–voltage curves caused by the pump current development were investigated. The pump current was found to be potential-dependent. It decreased with increasing hyperpolarization of the neurone.

3. With large hyperpolarizations the current–voltage curves obtained before the sodium injection and after eliciting the pump current coincided with each other. An increase in the membrane conductance was observed over the range of membrane potential corresponding to the pump current display.

4. The applied sodium injections did not cause any marked changes in the passive permeability of the membrane. This fact made it possible to measure the charge transferred across the membrane during operation of the pump current. Unlike previous data, the ratio of this value to the charge used to inject sodium into the neurone appeared to be a variable.

5. When the preparation was cooled to $+11°$ C, and also during the first few minutes after the application of a potassium-free solution, both the pump current and the membrane potential at which it disappeared could increase.

6. The pump current measurements during a number of transitions from one fixed level of the membrane potential to another showed that the

current did not depend upon the potential at which it developed before each transition.

7. The data presented allow the suggestion that the potential dependence of the pump current is determined by the changes in the rate of active transport of potassium, while the rate of active transport of sodium remains constant.

INTRODUCTION

The active transport of ions across the surface membrane may be accompanied by the appearance of an additional potential difference, as was shown first by Kernan (1962), and then confirmed by Cross, Keynes & Rybova (1965), Mullins & Awad (1965), Adrian & Slayman (1966) and others. Since then electrogenic transport has become the subject of growing attention, especially after it was also found in the nerves cells (Kerkut & Thomas, 1965; Ayrapetyan, 1969*a*, *b*).

A variety of reports have appeared suggesting that the effects of electrogenic transport may be observed not only after an excessive loading of the nerve cell with sodium (which leads to a marked intensification of the transport mechanism), but can also be a component of certain functional changes during its natural activity, such as post-tetanic hyperpolarization (Rang & Ritchie, 1968; Nakajima & Takahashi, 1966) or post-synaptic potentials (Libet & Kobayashi, 1968). The actual mechanism of the electrogenic effect during active transport still needs further clarification in many respects. Both the resting membrane potential of the cell, the nature of the ions transported, and the current flowing across its membrane are important.

Such measurements are possible under a long-lasting voltage clamp. This method has been successfully applied by Thomas (1969) to the investigation of the membrane current accompanying the electrogenic effects in an identified neurone of the snail. Combining a voltage clamp with measurements of the intracellular activity of sodium ions, Thomas found that the sodium injected into the neuronal soma caused an outward current proportional in size to the sodium ion activity inside the cell. The charge transferred by this current was about 28% of the charge transferred during the iontophoretic injection of the sodium.

The aim of the present study was to find out whether or not this current depended upon the membrane potential. The data obtained are somewhat different to those already published, and probably demand some modifications in our ideas about the principles of the electrogenic transport mechanism.

METHODS

The experiments were carried on giant neurones of the snail *Helix pomatia*. No special selection of identified neurones was made, but the largest cells were usually used. The method of preparation was the same as described by Krishtal & Magura (1970).

Ionic content of the Ringer solution was similar to that used by Kerkut & Thomas (1965). The pH was adjusted to 7·4 with Tris-HCl.

In the solutions with lowered potassium ion concentration, potassium chloride was replaced with sodium chloride. Ouabain (strophanthin G) or strophanthin K was added if necessary to the Ringer solution up to the concentration of 10^{-4} M.

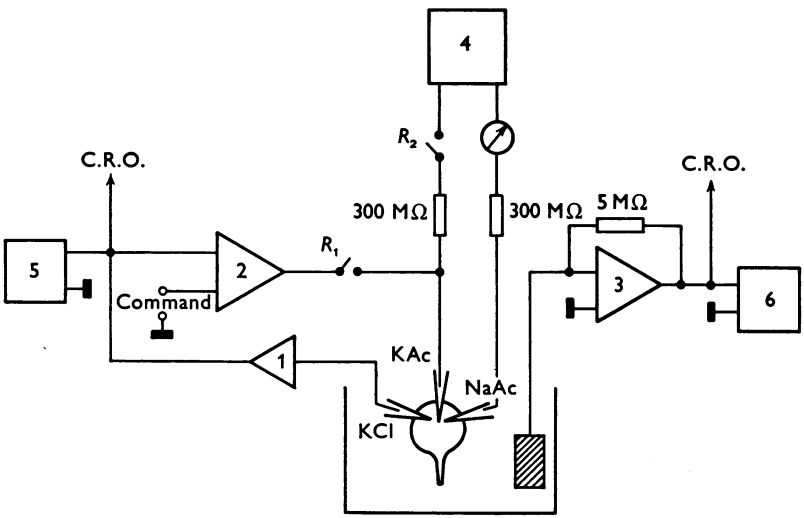

Fig. 1. The experimental set up for the long-lasting voltage clamp and intracellular iontophoresis: (1) the cathode follower; (2) the operational amplifier for voltage clamp; (3) the operational amplifier for current measurements; (4) the stimulator with the isolation unit at the output; (5, 6) pen recorders; R_1, the microrelay, closing the feed-back circuit for voltage clamp; R_2, the microrelay, closing the iontophoretical circuit.

Three separate micro-electrodes were inserted into the cell under investigation. The micro-electrode used for the potential measurement was filled with 2·5 M potassium chloride, that for intracellular iontophoresis of sodium ions with 1 M sodium acetate. The third electrode was used simultaneously as the second electrode in the iontophoresis circuit and in the feed-back circuit of the voltage clamp device. It was filled with 1 M potassium acetate (see Kerkut & Thomas, 1965). The resistance of the electrodes was usually 7–15 MΩ. A simplified diagram of the electrical system is shown in Fig. 1. The circuit for the injection was closed within the cell, so that the iontophoretic current caused neither polarization of the membrane nor artifacts in the feed-back current when the membrane potential was under control.

Owing to the isolating resistances (300 MΩ each), only 1–3 % of the feed-back current could pass through the micro-electrode filled with sodium acetate. The only inconvenience of such a circuit was the necessity for the potassium acetate-filled micro-electrodes to pass comparatively large currents.

The injection current was measured with a galvanometer (sensitivity 10^{-9} A). Two similar operational amplifiers were used for the voltage clamp and for the current measurements. Each of them had a gain of about 10^4. The potential base line drift measured at the output of the cathode follower did not exceed 0·5 mV/hr when the feed-back circuit was closed.

The small size of the membrane current to be measured demanded a strict control of the fluctuations in the feed-back current, which were due mainly to any fluctuations in the tip potential of the potassium chloride-filled electrode. Therefore such fluctuations were checked before each experiment. A passive resistance of 5 MΩ was inserted in the circuit instead of the membrane, and the feed-back circuit was then switched on. The potential recording micro-electrode was regarded as suitable if the amplitude of the low frequency fluctuations did not exceed 5×10^{-10} A. The experiments were done only with those neurones that had an input resistance greater than 2 MΩ; otherwise the amplitude of the current fluctuations could become comparable with the value of the phenomena to be investigated.

RESULTS

To increase the sodium concentration within the cell a direct current of $0{\cdot}4–1 \times 10^{-7}$ A was passed between the sodium acetate and potassium acetate-filled micro-electrodes. The process of iontophoresis was usually accompanied by an increasing hyperpolarization of the membrane. If the membrane potential was clamped at the initial level a corresponding increase in the feed-back current necessary to counteract the hyperpolarization of the cell was observed.

The charge carried by the sodium ions injected into the neurone (q_{inj}) could be calculated from the following equation:

$$q_{inj} = I_{inj} \times t_{inj} \quad (1)$$

were I_{inj} and t_{inj} are the iontophoretic current and the duration of the injection (it is assumed that the fluxes of ions passing from the cell into the injection micro-electrodes are negligible in comparison with the ion fluxes in the opposite direction, because the salt concentrations in the micro-electrodes are considerably higher than those in the cell). q_{inj} varied in our experiments from 0·5 up to 25 μC. Intracellular injections of the equivalent amounts of potassium (produced by an iontophoretic current in the opposite direction) did not cause any noticeable changes in the membrane potential (except a small depolarization; see Thomas, 1969). The latter fact was used to avoid any uncontrolled entry of sodium ions into the cell from the sodium acetate electrode due to diffusion or the leakage of part of the feed-back current (see Methods). A small direct current ($1–2 \times 10^{-9}$ A) was passed through the injection circuit all the time for this purpose, blocking the unwanted income of sodium, but loading the cell with potassium acetate at a negligibly low rate.

The sodium-induced increase in the feed-back current was inhibited by

ouabain or strophanthin K as it is illustrated in Fig. 2*B*. It must be noticed that both ouabain and strophanthin K in our experiments usually altered the conductance of the membrane (see Fig. 2*B*, the shift in the base line after the application of strophanthin K). Cooling of the preparation to +7° C also led to its disappearance (see below). These facts per-

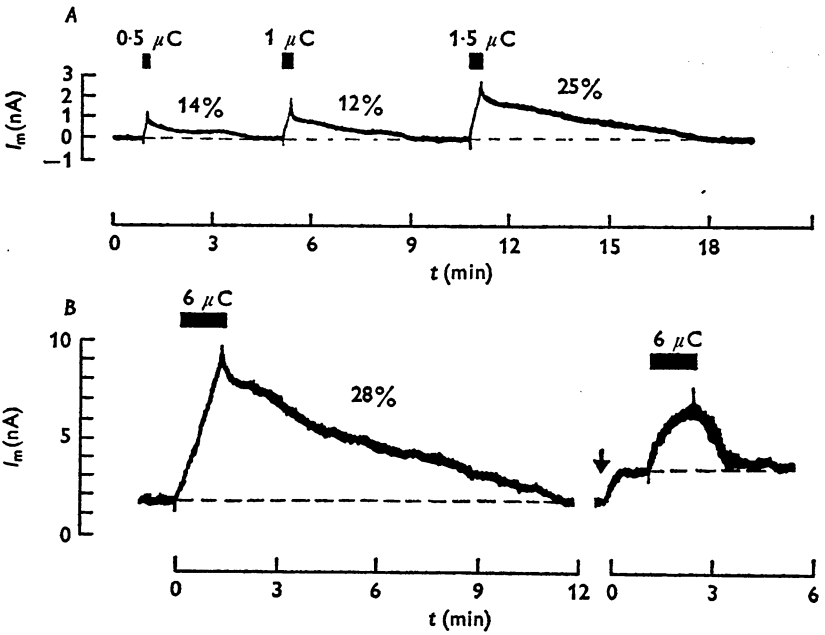

Fig. 2. The increase in the transmembrane feed-back current induced by the intracellular injection of sodium (I_{pump}). The membrane was clamped at a level of −43 mV. The duration of the injection here and on the following figures is marked with a black stripe. The charge transferred into the cell during the injection is indicated over the stripe.

A, I_{pump}, caused by three different intracellular injections of sodium. *B*, inhibition of I_{pump} by strophanthin K applied in the concentration of 10^{-4} M. Strophanthin K itself caused a slight hyperpolarization of the cell membrane, which can be seen from the shift in the steady level of the feed-back current, marked by the interrupted line.

The moment at which strophanthin K was applied is marked by the vertical arrow. The figures near the curves show the charge pumped out of the cell (calculated according to the eq. (2)) as a percentage of the charge carried into the cell during the iontophoresis (*A*, *B* are different neurones).

mitted the conclusion that the observed increase in the feed-back current was of the same nature as the 'electrogenic effect of the sodium pump' described by Thomas (1969) for the identified neurone of the snail, and could be classified as the 'electrogenic pump current'.

The charge carried by the pump current during the duration τ of the effect is given by

$$Q = \int_0^\tau (I - I_0)\,dt = \int_0^\tau I_{\text{pump}}\,dt, \qquad (2)$$

where I_0 is the initial value of the feed-back current and I its value at time t. Q increased with the increase in q_{inj}. However, the ratio Q/q_{inj} was found to be a variable which increased with q_{inj}. Thus in the illustrated case (Fig. 2*A*) $Q/q_{\text{inj}} \times 100\,\%$ was 12 % at $q_{\text{inj}} = 1\,\mu$C and 25 % at $q_{\text{inj}} = 1{\cdot}5\,\mu$C. The variations of Q/q_{inj} for different neurones were found to be even larger (1·5–70 %). A constant value close to 30 % found in previous experiments (Thomas, 1969; Kostyuk, 1970) is probably characteristic only for certain neurones of the snail. I_{pump} was investigated in this case only near the resting potential (at I_0 close to 0).

The injections of sodium ions were performed in our experiments at constant rates with an upper limit of 0·1 μC/sec. Higher rates of iontophoresis were not used in order to avoid any artifacts in the feed-back current. The majority of neurones demonstrated an approximately linear increase in pump current corresponding to a constant rate of sodium injection, at least when $q_{\text{inj}} \leqslant 5\,\mu$C. In some cases deviations from linearity were observed, in all cases the maximum of the pump current coincided with the end of injection. Considering, as postulated above, that the pump current reflects the action of the electrogenic sodium pump and is therefore sensitive to the intracellular sodium ion activity one may conclude the following: (*a*) the rate of increase in the internal sodium ion activity is close to the rate of increase in the internal amount of sodium, at least for the rates of the injection employed; (*b*) injection of up to 5 μC sodium forces the pump to operate at a rate which is still far from the saturation level.

To analyse whether the observed phenomena are accompanied by any changes in the membrane conductance a study of continuous current–voltage relations in the membrane was performed. With this purpose a command potential giving a constant rate of hyperpolarization was applied to the membrane in the voltage-clamp conditions. This procedure was repeated before the sodium injection and at different moments after it. Current–voltage curves were compared both when the pumping mechanism was blocked by cooling or ouabain and during the pump current development. The rate of the hyperpolarization (usually 0·25–2 mV/sec) was chosen to ensure: (*a*) the recording of a stable current–voltage curve for the resting membrane; (*b*) the recording of at least a quasi-stable current–voltage curve during the pump current development (this demand was probably fulfilled if pump current measured at a given holding

potential immediately before and after the hyperpolarizing shift showed inconsiderable changes); (c) a stability condition for the pump current measurement during a linear displacement of the membrane potential. Judging from the observation that the current–voltage curves of the membrane obtained at higher and lower rates of hyperpolarization coincided with those obtained at the chosen rates, those demands were fulfilled.

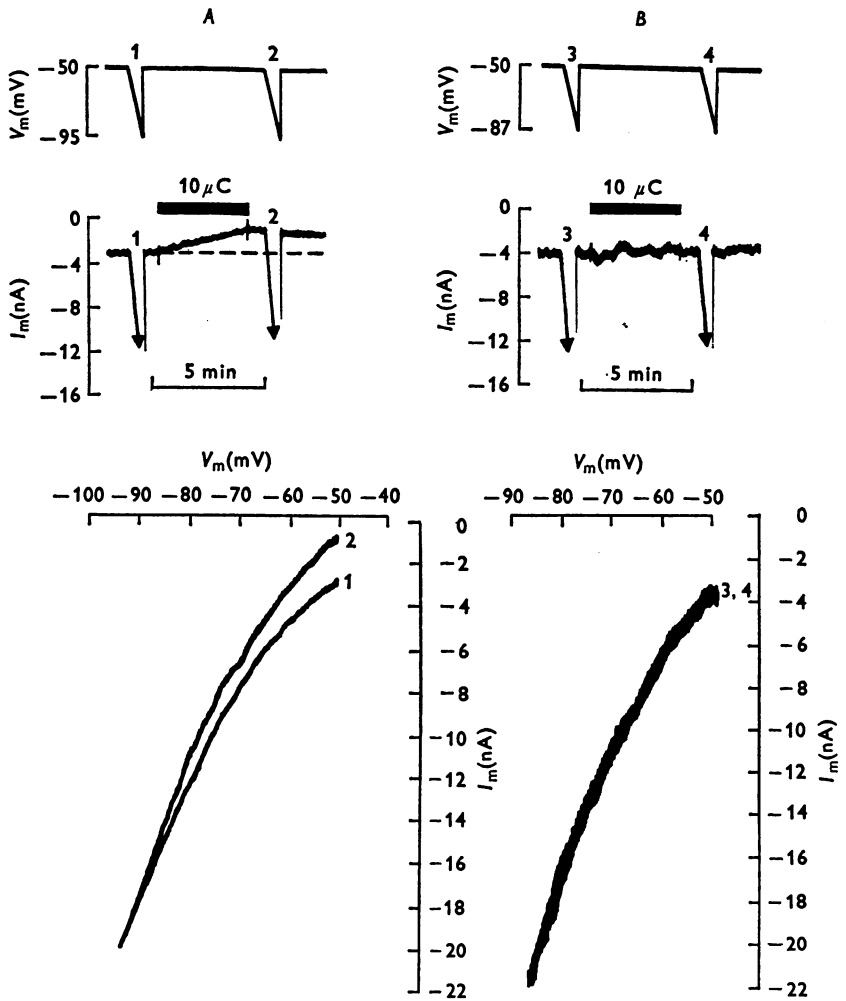

Fig. 3. The current–voltage curves obtained for one and the same cell after the sodium injection (10 μC) held in normal Ringer (*A*) and 5 min after application of ouabain (*B*).

Each column presents (from the top): membrane potential (voltage-clamp); feed-back current (curves broken with arrows); the superposition of the corresponding current–voltage curves.

Fig. 3 presents such curves obtained before and after sodium injection (10 μC) which was made first when the preparation was in normal Ringer (Fig. 3*A*) and then after the application of ouabain (Fig. 3*B*). Both pairs of curves were superimposed on a '*X*–*Y* plotter.' It can be clearly seen that the injection of sodium acetate does not produce any changes in the membrane conductance when the active transport mechanism is inhibited by ouabain. The same absence of changes in the current–voltage curves due to sodium injections was observed also under cooling to +7° C (see below).

Fig. 4 presents the effect of sodium injections in the conditions when the transport mechanism was unimpaired (21° C; normal Ringer). Fig. 4*A* demonstrates the time course of the pump current produced by a series of injections held one after another. The pump current increase followed an S-shaped curve. When the total amount of charges transferred into the cell was reaching 16·3 μC the increase in the pump current slowed down demonstrating the saturation effect. The current–voltage relations were measured in this experiment before the sodium injections and after each successive injection. The obtained curves (superimposed on the '*X*–*Y*' plotter) are presented in Fig. 4*B*. The effect was fully reversible.

The results of this experiment clearly demonstrate the following:

(1) Pump current (determined for a given V_m as a difference between the current–voltage curves obtained before and after the injection) is potential dependent. It declines monotonically while the hyperpolarization increases. At a certain level of membrane potential the pump-current disappears completely.

(2) Consequently, the membrane conductance (judging from the slopes of the current–voltage curves) increases with the pump current.

(3) The passive membrane permeability is not altered by sodium injections in conditions when the transport mechanism is activated.

The last conclusion is obvious from the fact that all the current–voltage curves coincided with sufficient hyperpolarization; they did not intersect irrespective to the amount of injected sodium.

The potential at which the pump current disappears under hyperpolarization will be denoted below as the 'pump current disappearance potential' (DP). DP varied from one neurone to another within the limits of −60 to −100 mV. q_{inj} at which saturation of the pump current could be achieved also varied (from 10 to 25 μC). Very large injections could produce insignificant changes in the passive permeability of the membrane, increasing membrane conductance.

In some neurones, however, sodium injections could produce changes resembling those expected if an additional source of current with a high internal resistance were switched on in the membrane. Fig. 5 demonstrates

an example of such a neurone. In this case, for moderate values of the hyperpolarization almost parallel current–voltage curves were obtained both before the sodium injection (Fig. 5, curve 1) and near the pump current maximum (curve 2). But with increasing hyperpolarization the

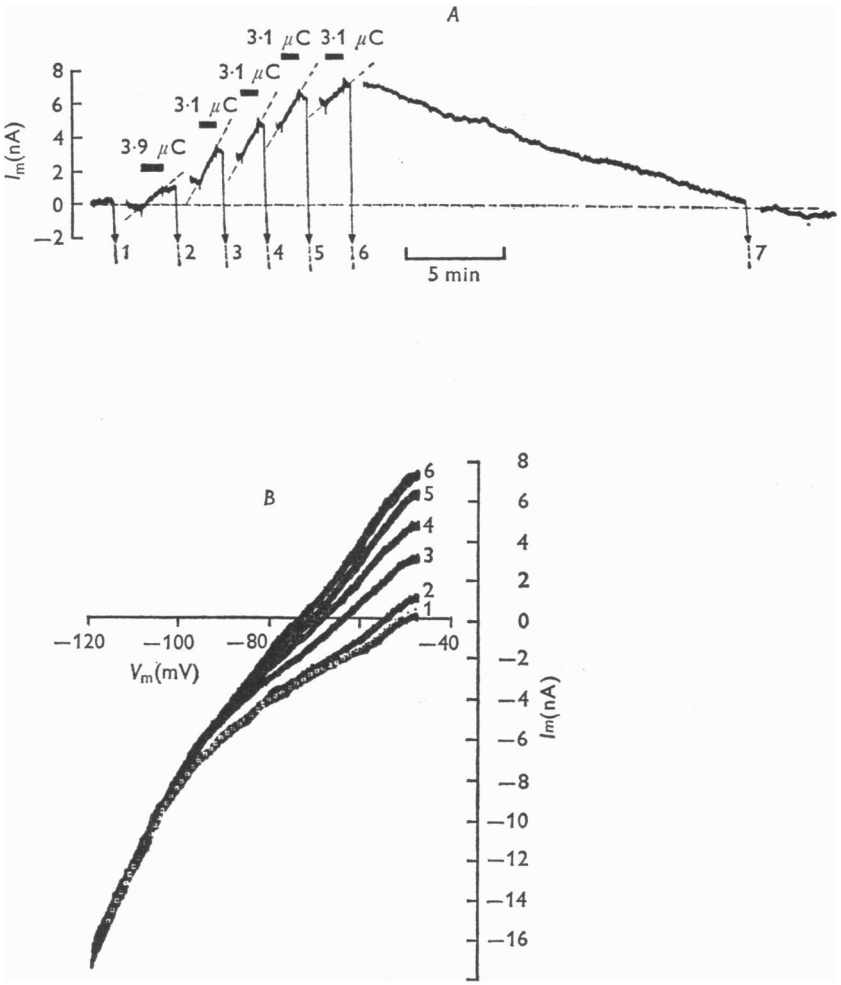

Fig. 4. The measurement of the dependence of pump current on potential (experiment held at a room temperature).

A, feed-back current. Current induced by a series of sodium injections. Total charge transferred to inject sodium was 16·3 μC. The slopes of the interrupted lines approximately show the rate of pump current rise during each successive injection. Current–voltage curves (numbered from 1 to 7) are broken with arrows.

B, the superposition of the current–voltage curves obtained in the experiment presented in *A*. The curve 7 almost coincides with the curves 1 and 2. It is marked with crosses.

curves gradually approached each other. Precise measurements of pump current in the region of deep hyperpolarization was difficult because a decrease in membrane resistance usually appeared, similar to the 'high conductance state' described by Marmor (1971*a*).

To find out whether the potential dependence of the pump current reflected the possible changes in the total rate of active transport of ions, the following experiments were performed. The pump current was

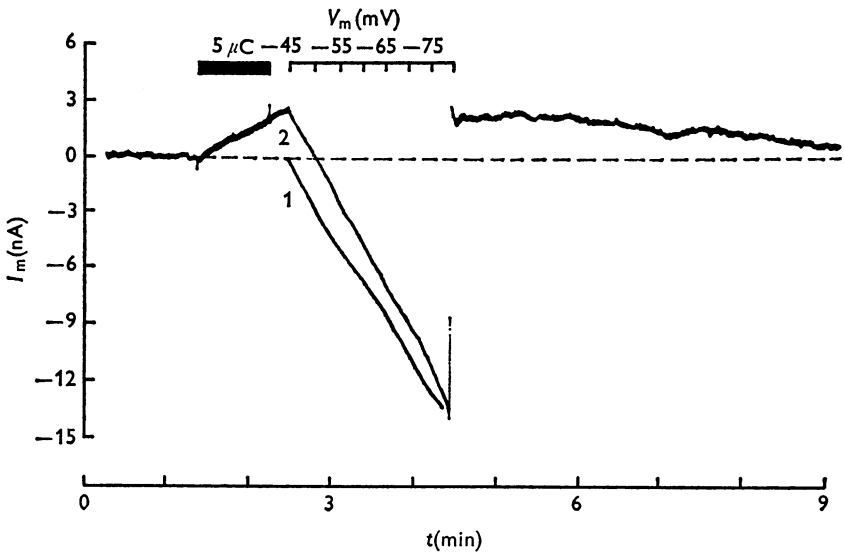

Fig. 5. The potential dependence of pump current for another neurone. The pump current disappearance potential was not reached, even at maximum hyperpolarization of the membrane, 1: the current–voltage curve, obtained before the sodium injection. 2: the same curve, obtained at the maximum value of pump current. The two curves are superimposed at corresponding membrane potentials, marked on the scale above the curves.

measured in a given cell for a series of the holding potential transitions between the two fixed levels. The result of such an experiment is demonstrated in Fig. 6. The sodium injection (17 μC) was first performed at a holding potential = −52 mV (upper curve) and then at a holding potential = −78 mV (lower curve). During the development of the pump current at a holding potential = −78 mV the membrane potential was returned from time to time to the previous level (holding potential = −52 mV).

Supposing that such shifts in holding potential while influencing the pump current also alter the total rate of the sodium transport, one would expect a different time course for the $[Na]_i$ decline for each level of the

membrane potential. It is obvious that in such a case, periodical returns of the holding potential from −78 to −52 mV would demonstrate values of the pump current different from those observed during the uninterrupted voltage-clamp at −52 mV. However, it was found that the pump current repeated exactly its time course at each transition of the holding potential. Obviously the pump current is determined at each moment only by the acting level of the membrane potential irrespective to the potential at which it developed before.

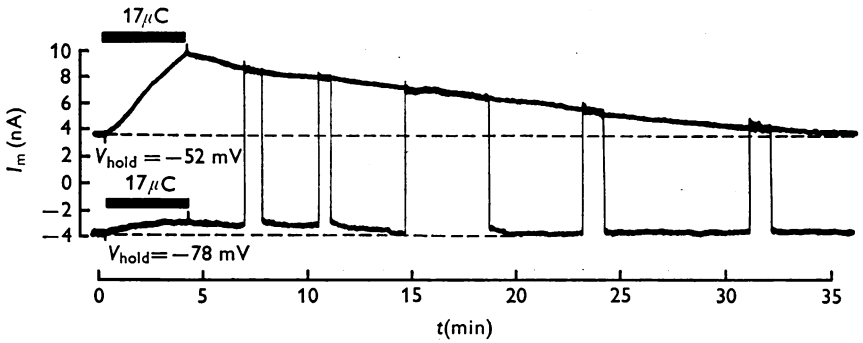

Fig. 6. Measurement of pump current at two different levels of the membrane potential; the sodium injections were the same in each case. Pump current curves obtained at a holding potential of −52 mV (upper curve) and a holding potential of −78 mV (lower curve). A series of returns to a holding potential of −52 mV were performed in the latter case.

The interdependence of pump current and holding potential was examined under the influence of temperature changes. The hysteresis-like curve was found for the temperature dependence of the resting potential. Fig. 7 shows the results of the experiment of such a kind. The preparation initially incubated at +19° C was cooled to +7° C, then rewarmed to +19° C again. All changes in the temperature were performed at maximum speed so as to avoid any possible loading of the cell with sodium ions that might take place as a consequence of cooling. The time during which the preparation was under the temperature below +10° C lasted only slightly over 3 min. Cooling to +7° C brought about 11 mV depolarization of the cell. Its conductance decreased from 0·35 μmho to 0·20 μmho. Rewarming was accompanied by a steeper temperature dependence of the resting potential. The level of −56 mV, corresponding in the initial incubation to +19° C, was already achieved at +12° C. When the temperature reached +19° C the cell was slightly hyperpolarized (about 3 mV). The initial resting potential was re-established only after almost 2 min incubation of the cell at +19° C. The conductance of the neurone measured during the rewarming exceeded that measured during cooling

at temperatures between +7 and +16° C (Fig. 7). But at higher temperatures the corresponding conductances were altered during rewarming in almost exactly the same manner as during cooling.

Fig. 8 demonstrates the effects of sodium ion injections carried out at various temperatures (holding potential = −40 mV and q_{inj} = 5 μC were constant). In view of the above mentioned features of the temperature

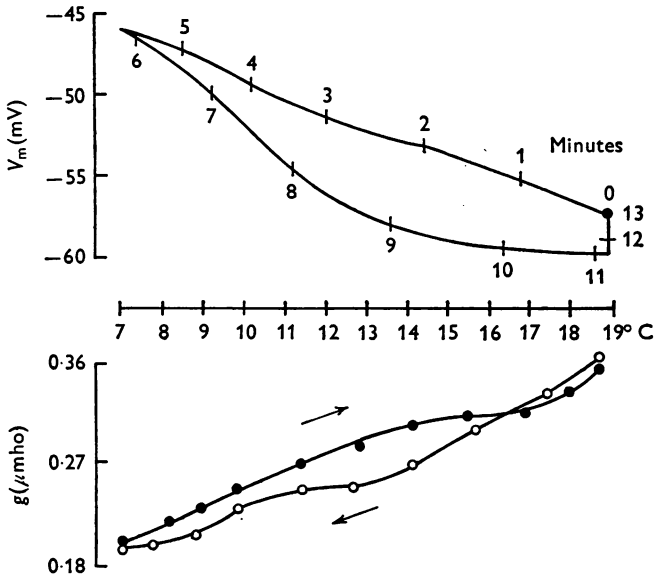

Fig. 7. The changes in the resting potential (upper curve) and in the conductance of the neurone (lower curve) due to quick cooling and subsequent rewarming. Vertical marks on the upper curve show the time from the beginning of the cooling.

dependence of the resting potential the preparation was incubated at a given temperature for 4–5 min before each injection. At 20° C the DP was −60 mV. Cooling to +11° C caused a sharp increase in the DP, up to −90 mV. But further cooling (to +7° C and lower) led to a total inhibition of the pump current.

Fig. 9 illustrates a similar experiment performed on another neurone. Besides the current–voltage curves obtained at 11–12° C, 18–19° C and 24–25° C the corresponding curves of pump current relating to V_m are shown (Fig. 9*b*). They distinctly show the effect of the temperature upon pump current, which (for a given V_m) and the absolute value of the DP considerably increase with cooling of the preparation from the room temperature to +11° C. In some cases cooling of the preparation could not produce any increase in the value of the pump current, but its potential dependence always weakened.

The exact behaviour of the pump current at temperature between +7 and +11° C remains uncertain since any small hardly measured deviations in the temperature could lead to the considerably differing values of current produced by the sodium injections of the same size into one and the same cell.

The effect of a potassium-free solution on the pump current was found also to be quite complicated. In most cases the application of the potassium-free solution increased pump current (for a given holding potential)

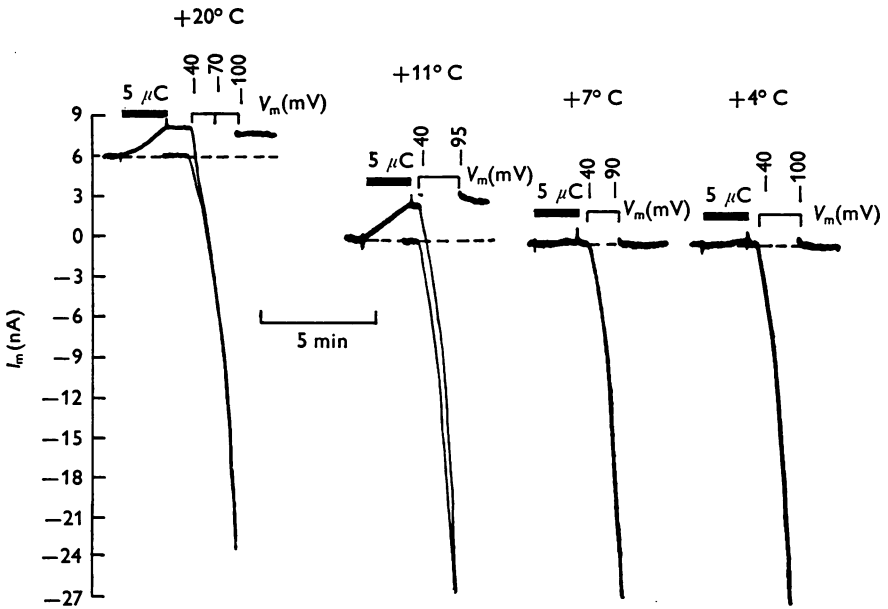

Fig. 8. The temperature dependence of pump current measured at 20, 11, 7 and 4° C. Pump current was produced by equal injections of sodium (5 μC). The current-voltage curves obtained before each injection and at the maximum of pump current are superimposed.

but after 10–20 min the pump current could not be produced at all by any additional injection of sodium. Immediate inhibition of the electrogenic effect by the potassium-free solution (see Thomas, 1969) was observed only in a few cases. Fig. 10 presents an experiment in which the pump current was elicited by injections of 10 μC of sodium. The injections were performed when the preparation was in the normal solution ($[K^+]_o$ = 6 mmole/l., Fig. 10*a*) and then after 8 and after 20 min in the potassium-free solution (Fig. 10*b*). The first sodium injection in the potassium-free solution still caused a considerable pump current, but a similar injection done only a few minutes later failed to induce any additional current.

Fig. 10*c* presents the corresponding curves for the pump current against

the holding potential, demonstrating an increase in pump current and its DP during the initial period after the application of the potassium-free solution. This effect appears to be similar to the effect of a moderate cooling (down to +11° C).

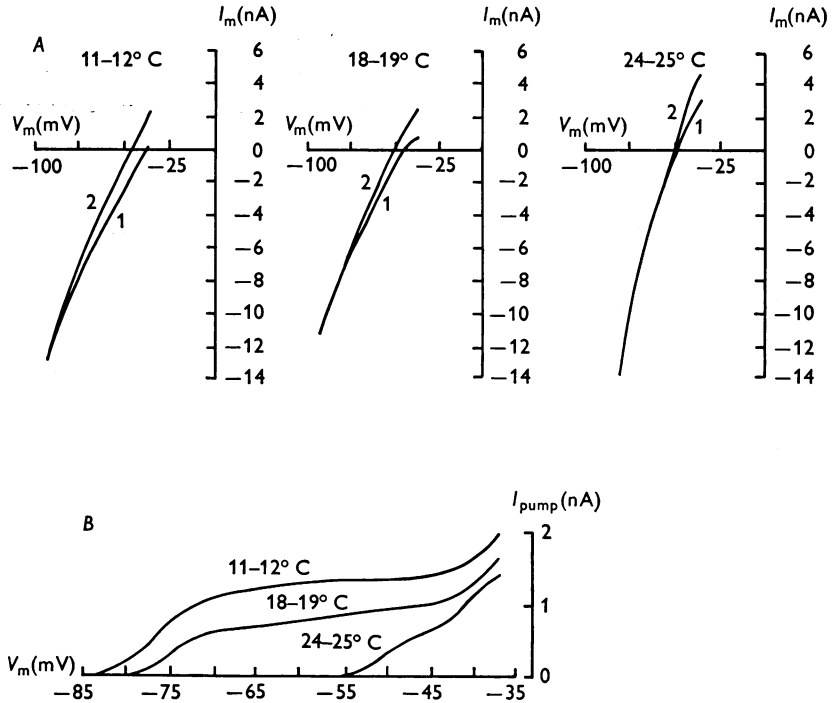

Fig. 9. The temperature dependence of the pump current. *A*, current–voltage curves, obtained at temperatures of 11–12°, 18–19° and 24–25° C. In each Figure two curves are superimposed, obtained before the sodium injection (1) and at the pump current maximum (2). *B*, the curves for the pump current against V_m at the different temperatures, obtained by the subtraction of curves *A* 1 and *A* 2.

DISCUSSION

The effect of an increase in the internal sodium ions in our experiments was qualitatively similar to that described before. The injection of sodium into the neurones led to their hyperpolarization (see Kerkut & Thomas, 1965). If the membrane potential was clamped at a fixed level the additional outward current appeared (see Thomas, 1968, 1969).

The hypothesis that the pump current was due to the process of active transport was well supported by several standard tests such as (*a*) application of ouabain, (*b*) cooling of the preparation, (*c*) removal of the external potassium ions. Each of these influences effectively inhibited the pump

current. Sodium acetate, when injected in a moderate amount into the cell which was affected by ouabain, low temperature or potassium-free solution, did not cause any noticeable changes in the current–voltage curve of the membrane (see Fig. 3; Fig. 8, curves corresponding to +7° C and +4° C; Fig. 10*b*, curves 5, 6).

Recently it has been shown by Marmor (1971*a*, *b*) that the anomalous rectification in the neurones of *Anisodoris* could be blocked by cooling and by potassium-free solution. It could therefore simulate the effect connected with the active transport process. In our experiments the

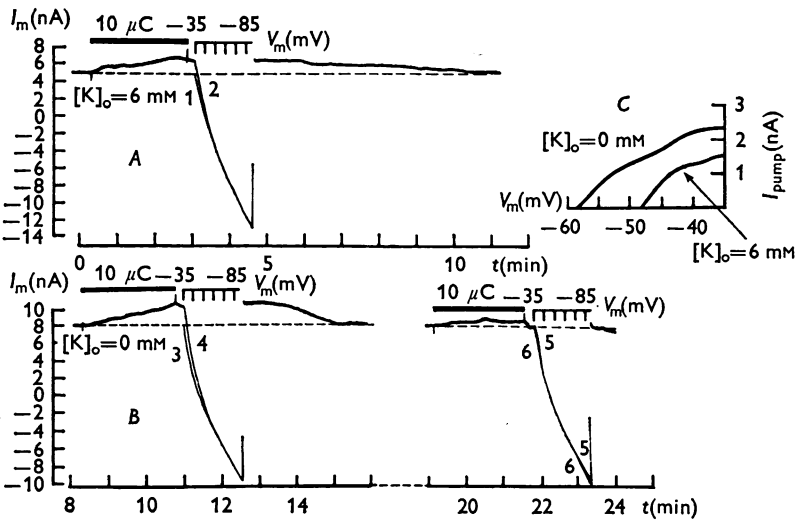

Fig. 10. Influence of a potassium-free solution on the pump current. Equal sodium injections (10 μC) were performed in the normal solution (*A*), and at different moments after it was replaced by the potassium-free solution (*B*). In each case the current–voltage curves obtained before the injection and at the maximum of pump current are superimposed. (*C*), the pump current against V_m curves, obtained by the subtraction of curves 1 and 2, 3 and 4.

anomalous rectification was also detected and it was to some extent sensitive to temperature (compare the curves presented at Fig. 9*A*). But the potential dependent pump current could be easily differentiated from this effect. Furthermore, in the cells where anomalous rectification was absent or even reversed, the potential dependence of the pump current remained (Fig. 10).

The first question to be posed is whether this additional current represents a direct product of the electrogenic $Na^+ - K^+$ pump or whether it is due to the membrane conductance changes probably accompanying the changes in the rate of the pump activity (Geduldig, 1968; Akiyama &

Grundfest, 1971). Our experiments have shown that the appearance of the pump current was accompanied by a conductance increase. However, the comparison of the continuous current–voltage curves obtained before each sodium injection and after its end (that is at a maximum pump current) demonstrated that any possible hyperpolarization of the membrane (sometimes even up to its electrical destruction at about -120 to -150 mV) could not cause an intersection of these current–voltage curves. All successfully investigated neurones demonstrated a monotonic decrease in pump current under the increasing hyperpolarization. Since no inversion of the sign of pump current could be achieved, the suggestion that the pump current was due to an increase in the passive permeability of the membrane coupled with the pump activity may be excluded (one may speculate that the proposed permeability increase is highly non-linear and very quickly diminishes with hyperpolarization, but such an idea seems to be hardly probable.)

The next problem to discuss is whether the data presented can give any information about the ionic basis of pump current. There are two basic concepts explaining the electrical effects directly accompanying the active transport of ions. According to one of them the Na^+–K^+ pump is electrically neutral (Na/K = 1) and the hyperpolarization following its activation is due to a decrease in the K^+ concentration near the outer surface of the membrane (Ritchie & Straub, 1957). On another hypothesis, a pump may act with a coupling ratio Na/K greater than 1 (Post & Jolly, 1957; Kernan, 1962). The results of the present investigation are in a better agreement with the second concept. It is difficult to regard the pump as electrically neutral because of (*a*) the potential dependence of the pump current, (*b*) the increase in pump current under moderate cooling, (*c*) the increase in pump current as the initial effect of the potassium-free solution. During the first minutes after application of the potassium-free solution a small amount of potassium ions, possibly sufficient for a partial functioning of the pump, may be detained in some extracellular storage space or gradually lost by the glial cells. However, it is well known that the resting potential of molluscan neurones depends only weakly upon the $[K^+]_o$ in the low concentration range (Gerasimov, Kostyuk, Maiskii, 1965; Moreton, 1969). Consequently it may hardly be supposed that the action of a neutral Na^+–K^+ pump would be able to cause any considerable hyperpolarization of the membrane when $[K^+]_o$ was already low. So the suggestion that the pump current is one directly produced by the electrogenic action of the Na^+–K^+ pump seems to be more preferable. In other words if $[Na^+]_i$ is higher than some 'normal' value, the total membrane current I_m may be expressed as a sum:

$$I_m = I_{passive} + I_{pump} \qquad (3)$$

where $I_{passive}$ is a current due to the passive permeability of the membrane.

An increase in the membrane conductance accompanying the appearance of pump current has an obvious relation to the potential dependence of the latter. As far as the pump current $(I)_{pump}$ is an increasing function of the membrane potential

$$\left(\frac{dI_{pump}}{dV_m} > 0\right)$$

the total conductance of the membrane also appears to increase:

$$g_m = g_{passive} + g_{pump}. \tag{4}$$

(It must be noted that although g_m becomes altered with an increase in $[Na^+]_i$ the evaluation of the total charge transferred by pump current according to the eqn. (2) is possible as $g_{passive}$ remains constant.)

The potential dependence of the pump current may be due to the following reasons:

(1) the changes in V_m alter both the rate of sodium extrusion and the rate of potassium intrusion in the same proportion (transport ratio Na/K remains constant):

(2) the changes in V_m alter the rate of active transport of only one ion (transport ratio Na/K alters);

(3) the changes in V_m affect the rate of active transport of both sorts of ion, but in different proportions (transport ratio Na/K also varies).

The experimental conditions of the present investigation did not allow direct measurements of the active fluxes of sodium and potassium ions. By relying on the experimental results demonstrated in Fig. 6, a preliminary choice between the three alternatives can be made. These results indicate that (*a*) the rate of the active extrusion of sodium ions from the neurone is independent of the observed electrical effect, (*b*) at least for $V_m = -40$ to -80 mV the rate of sodium extrusion does not depend upon the membrane potential (or only depends insignificantly). So the potential dependence of pump current must be determined by changes in the rate of potassium transport. Consequently the alternative (2) appears to be the more preferable.

It has been mentioned above that the pump current reached its maximum value near the end of the sodium injection. That caused by a given injection usually lasted much longer than the injection itself. It may therefore be suggested that:

(*a*) diffusion of the injected sodium throughout the volume of the cell was quite quick, and (*b*) the total rate of active extrusion of sodium (electrogenic plus neutral) was much lower than the rate of its iontopho-

retic intrusion (maximum 0·1 μC/sec). Under such circumstances it is possible to consider the time course of the pump current during the injection at a constant rate as an approximate curve $I_{pump}([Na^+]_i)$ for a given holding potential. This time course (for the small injections; far from saturation) was approximately linear, proving that the transport ratio Na/K was constant under these conditions. But if the ratio Na/K were really constant for a given V_m the following consequences would be expected: (*a*) an approximate constancy of the ratio Q/q_{inj} for different values of q_{inj} (for a given neurone, and with the injection time much less than the duration of the pump current display), (*b*) an exponential decay of the pump current. However, the data obtained did not correspond to these expectations. An increase in q_{inj} led to an increase in Q/q_{inj} (approaching sometimes 60–70 %), and the time course of the pump current decay was usually slower than exponential.

The resulting contradiction may probably be explained by supposing that the process of extrusion of large amounts of Na from the cell induces a change in the state of the pumping mechanisms such that the transport ratio Na/K and/or its dependence upon the slowly decreasing $[Na^+]_i$ gradually alters.

The approximately linear increase in pump current during the injection of sodium may be explained if one considers that the injection time is too short to produce any noticeable change in the state of the pump, and therefore in the ratio Na/K.

Proceeding from the above mentioned suggestions (and especially referring to the suggestion (2)) pump current may be expressed as

$$I_{pump}(V_m) = I^a_{Na} - I^a_K(V_m) \qquad (5)$$

where I^a_{Na} and I^a_K are the fluxes of sodium and potassium against their electrochemical gradients. It is suggested that pump current decreases with hyperpolarization because I^a_K increases while I^a_{Na} remains unaffected. At the DP, I^a_K becomes equal to I^a_{Na}. With such a model, the potential dependence of I^a_K may be qualitatively explained by supposing that the supply of energy available for the uphill (inward) transfer of a potassium ion is limited. The hyperpolarization lowers the energy barrier for the potassium ions (reducing the electrochemical gradient) and thus increases I^a_K and decreases the pump current. As to the sodium ions, the energy supplies for their transfer are presumably larger, and the corresponding changes in the electrochemical gradient are therefore too small to affect I^a_{Na}.

The peculiar temperature dependence of pump current discovered in our experiments may be explained in a similar way. Moderate cooling inhibits probably both I^a_{Na} and I^a_K. But since I^a_K is comparatively

'energy poor' it is more subjected to such an inhibition and so the pump current may increase. Stronger cooling becomes able to inhibit I^{a}_{Na}, and the pump current disappears.

As it is shown in Fig. 10 pump current increases during the first minutes after the application of the potassium-free solution. The potassium ion concentration outside the membrane is still sufficient in this initial period, because of the reasons mentioned above, to maintain a certain degree of I^{a}_{Na}. But the increased gradient for potassium ions elevates the energy barrier for their transfer and inhibits I^{a}_{K} so that the pump current and its DP increase.

These suggestions about a relative independence of I^{a}_{Na} and I^{a}_{K} are consistent to some extent with the results obtained in the experiments on squid axon. It was shown that arginine phosphate or a high ATP/ADP ratio was needed for a normal coupling between the active fluxes of potassium and sodium (Caldwell, Hodgkin, Keynes & Shaw, 1960; De Weer, 1970).

The data presented here do not allow us to carry out any quantitative analysis of the observed phenomena. Thus the measurement of the effective values of the gradients for the main cations involved in the action of Na^+–K^+ pump is very difficult under the present experimental conditions. For example, $[K^+]_o$ can hardly be estimated when the pump is active because the existence of extracellular diffusion barriers may lead to a substantial decrease in the potassium ion concentration just near the outer membrane (see above). The qualitative data presented here may indicate, however, that the current produced by an electrogenic pump is an effective mechanism controlling the transmembrane potential difference. The potential dependence of the pump current probably provides a kind of a negative feed-back in the working of such a control.

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